IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**DEX-0253**
Inventors:	**Sun et al.**
Serial No.:	**10/016,157**
Filing Date:	**October 31, 2001**
Examiner:	**Smith, Carolyn L.**
Group Art Unit:	**1631**
Title:	**Compositions and Methods Relating to Colon Specific Genes and Proteins**

Declaration by Dr. Roberto Macina

I, Roberto Macina, hereby declare:

1. I was awarded a M.S. in Biology and a Ph.D. in 1990 in Molecular Biology from the University of Buenos Aires, Argentina. After obtaining these degrees, I spent four years at The Wistar Institute, University of Pennsylvania contributing to the Human Genome Project endeavor. From 1995 to 1997, I served in the Molecular Diagnostic Department at SmithKline Beecham holding the positions of Investigator and Senior Investigator. Since the inception of diaDexus, Inc. in 1997 I have served as the Assistant Director of Cancer Gene Discovery. In October 2001, I assumed the position of Director of Molecular Technologies at diaDexus, Inc.

2. As the Director of Molecular Technologies, I am familiar with the teachings of the above-referenced patent application.

3. Since filing of the above-referenced patent application, the Gene Discovery division at diaDexus, Inc. has performed experiments following the procedures disclosed in the application

that confirm the utility of SEQ ID NO:5, also known as Cln237v1, with regard to colon cancer.

4. The relative expression analysis for SEQ ID NO:5 was performed in accordance with a standard Quantitative Polymerase Chain Reaction (QPCR) protocol well known to those skilled in the art at the time the application was filed. The procedure is described in the above referenced patent application at page 42, line 31 through page 43, line 19, page 96, lines 5-11, and Example 2 at pages 121-124.

5. I personally supervised experiments to measure the relative levels of SEQ ID NO:5 in cancerous, normal-adjacent, and normal tissues. In these experiments, relative quantitation of gene expression was done using Polymerase Chain Reaction in real time.

Quantitative PCR with fluorescent TAQMAN® probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (TAQMAN®) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA USA). Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT)) efficiency. Either cyclophilin, glyceraldehydes-3-phosphate dehydrogenase (GAPDH), ATPase, or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the comparative method (User Bulletin #2:ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of mRNA expression of the target gene are evaluated for every sample in normal and cancer tissues. Total RNA is extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA is prepared with reverse transcriptase and the polymerase chain reaction is done using primers and TAQMAN® probes specific to each target

gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

One of ordinary skill can design appropriate primers. The relative levels of expression of SEQ ID NO:5 versus normal tissues and other cancer tissues can then be determined. All the values are compared to the calibrator. Normal RNA samples are commercially available pools, originated by pooling samples of a particular tissues from different individuals.

The relative levels of expression of SEQ ID NO:5 in pairs of matched samples may also be determined. A matched pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual. All the values are compared to the calibrator.

6. Results from this QPCR experiment are depicted in the attached expression table. The table includes the Sample Name, which contains the tissue and ID of the sample, (e.g. CLNAS12 is Colon sample AS12), and the expression value for the sample in cancer tissue, Normal Adjacent Tissue (NAT), Normal tissue, Benign Prostatic Hyperplasia (BPH) or Prostatitis (Prost) as appropriate. All expression values are relative to the normal colon sample CLN01CL (calibrator) which is given an expression value of 1.00.

The values in the table show that the expression levels of SEQ ID NO:5 are higher in the cancer samples tested when compared with all the normal tissues and the normal adjacent tissues for colon cancer. For example, samples CLNB34, CLN401C, CLNAS98, CLNCM12, CLNDC19, CLNRC01, CLNSG27 and CLNTX01 all have higher expression levels in cancerous tissue than in normal adjacent tissue in the same sample, as well as higher expression levels than in the colon normal tissue sample CLN01CL.

The sensitivity calculated comparing the levels of SEQ ID NO:5 in the colon cancer samples versus the expression in the colon normal adjacent tissue from the same patient is **60%** (meaning that **60%** of the cancer samples show levels of SEQ ID NO:5 higher than the corresponding normal adjacent tissues from the same patient). The specificity for SEQ ID NO:5 for colon is **62%**. This specificity an indication of the level of colon tissue specific expression of the gene compared to all the other tissue types tested in our assay. Thus, these experiments confirm the

utility of SEQ ID NO:5 with regard to colon cancer.

7. I understand that the Examiner has taken the position that nucleic acid molecules comprising SEQ ID NO:5 lack a specific and substantial utility, and therefore would not be useful as a diagnostic for colon cancer. As demonstrated in the instant application and confirmed in the experiment discussed herein, SEQ ID NO:5 is differentially expressed in colon cancer tissue versus normal tissue and normal adjacent colon tissue. Many FDA approved and commercially available therapeutic and diagnostic products are based on the differential expression of molecules in cancer tissue versus normal tissue. For example, Genentech's therapeutic product Herceptin® and its diagnostic counterpart, the HercepTest® are very successful commercially, yet many publications show the relevant gene, HER-2 is overexpression in only 30% of breast cancer patients.

Consequently, the measurement of SEQ ID NO:5 is at least as specific and sensitive as commercial cancer diagnostics on the market today. Therefore, in my professional opinion the specificity and sensitivity of SEQ ID NO:5 is sufficient for use with regard to detecting colon cancer.

Moreover, nucleic acid molecules comprising SEQ ID NO:5, vectors and host cells are useful as standards and/controls for a diagnostic assay (whether detecting the nucleic acid or a protein encoded thereby). In addition, nucleic acid molecules comprising SEQ ID NO:5, vectors and host cells are useful to make and validate a therapeutic monoclonal antibody targeting cells over-expressing the protein encoding by SEQ ID NO:5. One would also use such materials in the preparation and testing of a vaccine or antisense molecule which targets cells over-expressing SEQ ID NO:5.

In conclusion, the differential expression of SEQ ID NO:5 demonstrates a credible, specific and substantial utility for colon cancer diagnostics or therapeutics.

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful statements and the like so made are punishable by fine or by imprisonment, or both, under §1001 of Title 18 of the United States code, and that such willful statements may jeopardize the validity of the

application, any patent issuing there upon, or any patent to which this verified statement is directed.

Roberto Macina, Ph.D.

12/22/04
Date

DEX-0253 SEQ ID NO:5 QPCR Expression Values Table

Sample	Can	NAT	Norm	BPH	Prost
CLNAS12	0.54	0.43			
CLNAS46	0.52	0.33			
CLNB34	3.67	0			
CLNCM67	0	2.12			
CLNDC22	0.64	2.21			
CLNTX89	0	0			
CLN401C	26.93	21.88			
CLNAS43	1.68	6.27			
CLNAS98	9.09	7.59			
CLNCM12	27.87	2.98			
CLNDC19	15.8	4.15			
CLNRC01	18.07	11.22			
CLNRS53	31.21	33.93			
CLNSG27	33.23	20.56			
CLNTX01	27.8	5.41			
BLD030B	0.36	4.11			
BLD520B	1.05	0.73			
BLDTR17	2.65	2.7			
CVXKS52	0	0.81			
CVXNKS55	0.09	0.15			
CVXNKS25	3.88	1.8			
CVXNKS18	3.21	0.27			
CVXNKS54	0.87	6.56			
ENDO10479	5.41	2.42			
ENDO28XA	0	0			
ENDO8XA	0	0			
KID106XD	0	0			
KID12XD	0	0			
KID10XD	0	0			
KID22K	0	0			
KID107XD	0	0			
LNG205L	2.74	2.33			
LNG315L	0	0.4			
LNG507L	0	0			
LNG528L	0.23	1.66			
LNG8837L	0	0.64			
LNGAC11	2.58	0.92			
LNGAC39	0.42	0.57			
LNGSQ80	0.08	0.11			
LNGSQ81	0.23	13.21			
LVR15XA	0	0			
LVR174L	0	0			
LVR187L	0	0			
MAM19DN	0	0			
MAM42DN	0	0			
MAM517	0	0			
MAM781M	0	0			
MAM869M	0	0			
MAM976M	0	0			
MAMS570	0	0			
MAMS699	0	0			
MAMS997	0	0			
OVRG021	0	0			
OVR206I			0		
OVR515O			0		
OVR18GA			1.08		
OVR337O			0		
OVR123O			4.95		
OVRC177			0.1		
OVR40G			0.39		
OVR1005O	0				
OVR1040O	0.11				
OVR105O	0.74				
OVR130X	0				

Sample	Can	NAT	Norm	BPH	Prost
OVR451O			6.84		
OVR718O	0.27				
OVRA1B	0.68				
PAN71XL	0	0			
PAN77X	0	0			
PAN92X	1.14	0			
PRO10R					2.42
PRO20R					1.77
PRO23B	2.9	3.44			
PRO263C				0	
PRO276P				2.84	
PRO65XB	0.51	6.91			
PRO675P	0.78	3.74			
PRO767B				2.47	
PRO84XB	1.94	9.34			
PRO855P				6.38	
PRO958P	2.19	1.24			
SKN287S	0	4.35			
SKN39A	0	1.67			
SKN669S	9.24	3.92			
SMINT171S	2.83	5.41			
SMINT20SM	3.69	33.83			
SMINTH89	0.3	2.01			
STO261S	25.65	18.06			
STO288S	85.4	0			
STOAC93	3.31	10.11			
STO88S	74.48	0.29			
THRD143N	0	3.79			
THRD270T	0	0.21			
THRD56T	1.12	0.34			
TST39X	5.93	0			
TST647T	1.11	0			
TST663T	4.69	0.57			
UTR135XO	4.04	0.63			
UTR85XU	24.26	24.24			
BLOB3			3.1		
BLOB11			0		
BLO69			0		
BLO72			0		
BLO73			0		
ADR48AD			1.74		
BRN10BR			0		
CLN01CL			1		
CVX06CV			6.28		
ESO01ES			2.94		
HRT46HR			0.03		
HUMREF00HR	2.13				
KID55KD			0.1		
LVR89LV			0		
LNG90LN			0.01		
MAM01MA			0.03		
MSL84MU			0		
OVR3APV			0		
PAN04PA			0.02		
PLA59PL			4.61		
PRO09PR			0.47		
REC21RC			5.1		
SMINT59SM			4.59		
SPL7GSP			0.27		
STO09ST			0		
THYM99TM			0		
TRA16TR			2.8		
TST4GTS			2.17		
UTR57UT			1.97		